

## LABORATORY ANIMAL PROJECT REVIEW

**Please note:**

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

### LAPR Information

**LAPR Title:** To develop a new technique to determine heart rate characteristics in rat with ECGenie System  
**LAPR Number:** 20-08-002  
**Principal Investigator:** Exemption 6  
**Author of this Document:** Exemption 6/RTP/USEPA/US  
**Date Originated:** 07/19/2017  
**LAPR Expiration Date:** 08/31/2020  
**Agenda Date:** 07/26/2017  
**Date Approved:** 08/07/2017  
**Date Closed:**

### APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US b)Exemption 6/RTP/USEPA/US	08/07/2017	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US b)Exemption 6 /RTP/USEPA/US	08/07/2017	DMR	


### Administrative Information

**1. Project Title (no abbreviations, include species):**

To develop a new technique to determine heart rate characteristics in rat with ECGenie System

**Is this a continuing study with a previously approved LAPR?**

No

**2. Programatic Information**

**a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.**

SHC Project 2.63: Assessing environmental health disparities in vulnerable groups; Task 2: Interactions between chemical stressors and social factors that impact children's health and development

**b. What is the Quality Assurance Project Plan (QAPP) covering this project?**

QAPP development pending on training on use of new apparatus

**3. EPA Principal Investigator/Responsible Employee:**

<b>Principal Investigator</b> <b>Exemption 6</b>	<b>Phone Number</b> <b>Exemption 6</b>	<b>Division</b> TAD	<b>Mail Drop</b> MD
	<b>Lotus Notes Address</b> <b>Exemption 6</b>	<b>Branch</b> DTB	B105-04
	Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US		

**4. Alternate Contact:**

<b>Alternate Contact</b> <b>Exemption 6</b>	<b>Phone Number</b> <b>Exemption 6</b>	<b>Division</b> TAD	<b>Mail Drop</b> MD
	<b>Lotus Notes Address</b> <b>Exemption 6</b>	<b>Branch</b> DTB	B105-04
	Exemption 6 Exemption 6 Exemption 6 Exemption 6 S/RTP/USEPA/U		

### SECTION A - Description of Project

**1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the**

**continuation. Please spell out all acronyms and abbreviations with their initial use.**

A recent hypothesis for public health suggests developmental origins of health and diseases, in that many metabolic diseases (such as congestive heart failure and diabetes) may be originated from altered nutritional status and chemical insults during embryonic and fetal development. In other words, unbalanced food intake, exposure to stressful environment and noxious chemicals during pregnancy can make the offspring more vulnerable to diseases and ill-health later in life. Such hypothesis, if proven, will influence the current practice of human health risk assessment. The objective of our study is to use a laboratory rodent model to determine whether maternal stressors (such as high-fat diet and psychosocial stress) may interact with early life exposure to environmental pollutants to produce latent adverse health outcomes. Such effort will require longitudinal evaluation (from neonates to adults) of the impacts of chemical and non-chemical stressors on physiological functions. To determine cardiac performance such as heart rate in rodents, electrocardiography (ECG) is typically performed under anesthesia or with surgical implantation of a radio-transmitter. However, such techniques are unfeasible for longitudinal and repeated measures. A new non-invasive instrument has recently been developed to record electrocardiograms in conscious small animals (from rat and mouse pup to young adult). This procedure entails placing the free-moving animal on an elevated recording platform, and the ECG signal will be acquired when three of four paws of the animal are in contact of the platform surface. The recording time typically is within 1-2 minutes, and the testing animal can be returned to its home cage. No pain or shock, will be introduced by the equipment, and no anesthetics, surgery or restraint is required. This LAPR requests several rats to allow training provided by the manufacturer staff. For details of the procedures, please see the following link: <https://vimeo.com/66169528>.

**2. Scientific rationale for proposed animal use.**

**a. Why is the use of animals necessary?**

Physiological assessment of cardiac function can only be determined in whole animal.

**b. Justify the species requested:**

Rat and mouse are the typical animal models used in laboratory, their ECG signals largely resemble those in humans.

**3. How was it determined that this study is not unnecessary duplication?**

This is for training purpose and is not unnecessary duplication.

**SECTION B - In Vivo Procedures**

**1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.**

This experiment requires rats in our animal facility to allow the trainers from ECGenie manufacturer to demonstrate the technique to our laboratory staff, and for our staff to practice the technique.

**2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.**

6 rats should be sufficient for demonstration and practice.

**3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions ): Please enter numbers only.**

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	6	
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

**4. Does this LAPR include any of the following:**

- ☐ Restraint (>15 Minutes) ☐ Survival surgery  
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

**5. Category C procedures. Describe each procedure separately, include details on the following:**

**a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):**

none

**b. Survival Blood Collections (method, volume, frequency):**

none

**c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):**

This procedure entails placing the free-moving animal on an elevated recording platform, and the ECG signal will be acquired when three of four paws of the animal are in contact of the platform surface. The recording time typically is within 1-2 minutes, and the testing animal can be returned to its home cage. No pain or shock, will be introduced by the equipment, and no anesthetics, surgery or restraint is required.

**d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:**

none

**e. Breeding for experimental purposes (e.g. length of pairing, number of generations):**

none

**f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:**

Rats are to be housed in pairs, one will be tail-marked for identification. This request is for training purpose, no permanent record keeping is required. Each rat will be tested for multiple (not more than 5) times to allow hands-on training by staff. Each rat will be returned to home cage immediately after each test. The entire training session will not exceed 3 hours. At the termination of training, all animals will be return to the donor LAPR.

**6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).**

**a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):**

**b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):**

**c. Testing methods:**

**d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):**

**e. Describe how animals will be monitored (e.g., frequency of observations, by whom):**

**f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:**

**g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:**

**7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)**

**a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:**

**b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:**

**c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):**

**d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):**

**e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?**

☐ Yes ☐ No

**f. Identify any surgical procedures performed at other institutions or by vendors:**

**8. Humane interventions (for treatments/procedures in all categories).**

**a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.**

No expected issues, but will consult AV and follow the recommendation if any adverse effects are observed

**b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.**

Since this is for training and no chemical is involved, no adverse effects are expected. For emergencies, we will seek the expert opinions of AV.

**9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.**

N/A

## **SECTION C - Animal requirements**

**Describe the following animal requirements :**

**1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.**

**a. Animals to be purchased from a Vendor for this study:**

**b. Animals to be transferred from another LAPR:**

LAPR Number that is the source of this

LAPR # 20-02-001

6

transfer:

**c. Animals to be transferred from another source:**

**d. Offspring produced onsite (used for data collection and/or weaned):**

**e. TOTAL NUMBER of animals for duration of the**

6

LAPR

**2. Species (limited to one per LAPR):**

Rat(s)

**3. Strain:**

**Describe special requirements for animals with altered physiological responses**

(e.g., genetically altered, aged)

Any available strain will do.

**4. Sources of animals:**

Training colony (LAPR # 20-02-001).

**5. Provide room numbers where various procedures will be performed on animals:**

NHEERL animal facility **Exemption 6**

**6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.**

No

*Room Numbers:*

**7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)**

N/A

**8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.**

N/A

**9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)**

None

**10. Housing and Enrichment.**

*The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.*

Pair housed as per donor LAPR.

**SECTION D - Agents Administered to Animals**

**1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used. Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.**

None

**2. Describe compounds to be administered to animals.**

**a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.**

None

**b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.**

N/A

**c. Provide a statement regarding any safety precautions necessary for handling any of these materials.**

N/A

**NOTE:** Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

## **SECTION E - Personnel Training and Experience**

**1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.**

**Use this area to type in additional personnel information not available in the table drop-down lists:**

**Hint:** The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).

<b>NAME</b>	<b>ROLE</b>	<b>SPECIFIC RESPONSIBILITY</b>	<b>RELEVANT TRAINING</b>
Exemption 6	Principal Investigator	Design and oversee project	completed all NHEERL required training
Exemption 6	Associate Principal Investigator	Design and oversee project	completed all NHEERL required training
Exemption 6	Technical Staff	Conduct experiment	completed all NHEERL required training
Exemption 6	Technical Staff	Conduct experiment	completed all NHEERL required training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

## **SECTION F - Animal Breeding Colonies**

**This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.**

**Describe:**

- 1. Estimated number of breeding pairs and liveborn per year**
- 2. Breeding protocols and recordkeeping**
- 3. Methods for monitoring genetic stability**
- 4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR**

## **SECTION G - Euthanasia**

**1. When will the animals be euthanized relative to experimental procedures?**

Not planned, but in case of emergency or unforeseen situation, animals will be humanely euthanized either by animal care staff or AV

**2. Describe the euthanasia techniques:**

**Method(s):** [Euthanasia plus exsanguination](#)

**Agent(s):** [CO2](#)

**Dose (mg/kg):** [to effect](#)

**Volume:**

**Route:** [inhalation](#)

**Source(s) of information used to select the above agents/methods:**

[2013 AVMA Guidelines on Euthanasia.](#)

**3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).**

[N/A](#)

**4. Describe how death is to be confirmed.**

[Vital organ section](#)

**SECTION H - Disposition of Used and Unused Animals**

**Describe the disposition of any animals remaining after project completion.**

[Transfer back to donor LAPR](#)

**The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?**

☒ [Yes](#) ☐ [No](#)

**SECTION I - Assurances**

**1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.**

**2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.**

**3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.**

**4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.**

**5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.**

**6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.**

**7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.**

**8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.**

[\[Signature Line\]](#)

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	07/19/2017

**Submitted:** 07/19/2017

**Resubmitted:** 07/31/2017

### **Certification:**

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	Approval Date	Phone Number	Division	Mail Drop
Exemption 6	07/19/2017	Exemption 6 Lotus Notes Address	TAD Branch DTB	MD Submitted to Branch Chief for Approval 07/19/2017 04:34 PM
	by Exemption Exemption 6 Exemption 6 A/US RTP/USEP	Exemption Exemption Exemption 6 Exemption 6 A/US RTP/USEP		

### **ATTACHMENTS**



20-08-002 PI resp1.pdf

### **Actions**

*First Update notification sent: 06/28/2018*

*Second Update notification sent:*

*First 2nd Annual notification sent:*

*Second 2nd Annual notification sent:*

*1st Expiration notification sent:*

*2nd Expiration notification sent:*

### **History Log:**